



# Multi-decadal variation in cohort specific sex ratios and otolith increment growth characteristics of juvenile blue grenadier (*Macruronus novaezelandiae*)

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## ABSTRACT

This study presents inter-annual variability of otolith growth from 1993 to 2011 and cohort specific sex ratios from 1976 to 2011 in blue grenadier *Macruronus novaezelandiae* on the west coast of Tasmania. Length-at-age data highlighted that *M. novaezelandiae* females grow larger than males with mean length-at-age statistically diverging by three years of age and remaining separated thereafter. Statistical differences in both cumulative, and age specific otolith increment widths-at-age between sexes were detected at earlier ages, but these effects were variable and not consistent with the sexual dimorphism observed for mean length at age. No differences between sexes were evident for the ages 8–14 or 7–11 for cumulative increment widths or age specific otolith increment widths-at-age respectively. Inter-annual variation in mean otolith increment widths for the first five increment zones (juvenile zones) showed a trend of declining increment widths from 2006 to 2010. Inter-annual cohort sex ratio variation across year-of-birth was also significant displaying a similar trend to the first-year otolith increment growth profiles. Regression analysis of birth year sex ratio with mean otolith increment widths indicated a significant negative relationship for first year growth with increasing numbers of male offspring. The inter-annual variation in cohort specific sex ratio suggests that environmental factors may be involved with sex determination. When coupled with climate change these influences could have long term consequences for the fitness of the stock and corresponding flow-on effects into the stock assessment process and future management of the stock.

## 1. Introduction

Blue grenadier *Macruronus novaezelandiae* (Hector 1871) is an important commercial fish species for both the Australian and New Zealand fishing industries (Gunn et al., 1989). In Australian waters, they are found on the upper continental slopes from New South Wales to Tasmania and across the Great Australian Bight (Kenchington and Augustine, 1987; Kailola et al., 1993). The species is targeted by both trawl and long-line fishers in the Southern and Eastern Scalefish and Shark Fishery, with the Commonwealth Trawl Sector accounting for most of the catch (Department of Sustainability, 2013; Georgeson et al., 2014). The Australian 2012/13 annual production was valued at \$15.5 million with a total landing of 3954 t (Georgeson et al., 2014). The fishery consists of two sub-fisheries, a non-spawning fishery operating throughout the year and a winter spawning fishery. The spawning fishery provides much of the Australian product (Kloser et al., 2007; Tuck, 2013) by targeting mature adults in the winter spawning

aggregation from July through to September off the western Tasmanian coast. Targeted winter trawling at depths of 300 m–600 m occurs over a slender 100 NM area between 41° 20' S and 42° 40' S. Trawls typically occur on the bathymetric features of the Pieman and Strahan Canyons and the Sandy Cape region with additional catches from the mid-slope regions within the area (Kloser et al., 2007; Ryan and Kloser, 2012; Tuck, 2013). Studies into the early larval history of *M. novaezelandiae* from the Tasmanian spawning ground suggest that larvae are passively advected by longshore currents to southern and eastern Tasmanian coastal waters, but also that the advection route is inter-annually variable (Thresher et al., 1988). Larvae disappear from the water column at about 55 days on the East coast of Tasmania (Lyne and Thresher, 1994). There are contradictory reports on where juveniles inhabit, some juveniles have been reported from near shore coastal environments and large estuaries (Wilson, 1981; Yearsley et al., 1999). Other studies suggest they inhabit the mid shelf depth ranges of 200–400 m (Kuo and Tanaka, 1984). How or when juveniles move

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between coastal or mid shelf habitats back to spawning areas in the species is poorly understood (Thresher et al., 1988). The lack of significant numbers of juveniles being captured by recreational fishers in near shore environments (Lyle et al., 2014), compared to the large mode of juveniles in trawl captures reported by Kuo and Tanaka (1984) suggests they may prefer to inhabit offshore environments.

Fish growth is a basic parameter underlying population characteristics such as stock biomass, population density and size frequencies (Jobling, 2002). Many investigators have found that growth rates are dynamic and vary across years, sometimes exhibiting distinct temporal trends (Thresher et al., 2007; Neuheimer et al., 2011). Variable growth rates in fish are influenced by intrinsic and extrinsic factors. Intrinsic influences on fish growth are species or individually explicit, such as age, sex and/or genetics (Weisberg et al., 2010; Morrongiello et al., 2012). For example, *M. novaezelandiae* are a sexually dimorphic species with females reaching larger sizes than males (Kailola et al., 1993; Smith, 1994; Froese and Pauly, 2014). Extrinsic influences differ because they exert external pressure on a fish's ability to grow and include aspects such as temperature, competition, food availability, habitat, and anthropogenic pressures for example, fishing effort (Thresher et al., 2007; Neuheimer et al., 2011; Morrongiello et al., 2012). Temperate fish species are very reactive to seasonal environmental factors (Gillanders et al., 2012) with growth variations being reflected in the width of individual growth zones within the otolith. The annual zones in *M. novaezelandiae* otoliths have been validated to form annually for ages two through 21 by modal progressions of age-classes (Robertson, unpublished). This technique has been previously used to validate ageing of this species in New Zealand waters (Horn and Sullivan, 1996). Wide translucent increments represent fast growth and narrow opaque increments represent slow growth (Francis and Campana, 2004; Bone and Moore, 2008; Green et al., 2009; Stocks et al., 2011). A combination of one translucent zone and one opaque zone represent one year of otosomatic growth. Increment widths near the otolith core are wider as juvenile otosomatic growth is relatively faster, but as the individual ages and matures, increment widths become progressively narrower. Variations in otolith increment widths have been previously used to provide insights into the factors influencing growth (Gillanders et al., 2012; Morrongiello et al., 2012). By examining variations in juvenile otolith increment widths, many of the confounding influences reproduction exerts on growth, such as reduced growth due to the attainment of maturity or growth effects influenced by inter-annual variations in reproductive activity and investment (Massou et al., 2004) can be eliminated. Few studies have been conducted on understanding inter-annual variations in otolith growth patterns in *M. novaezelandiae*. Therefore, the aim of this study was to investigate inter-annual variation in otolith growth patterns of *M. novaezelandiae* with a focus on juvenile otolith growth patterns from increment measurements. We also develop a 35 year time series of cohort specific sex ratios to test the hypothesis that they vary over time and present the correlation between juvenile otolith growth and cohort specific sex ratios.

## 2. Materials and methods

### 2.1. Data collection and growth modelling

Biological data and samples were collected by observers on-board commercial vessels while at sea, or at the ports of landing. Catches were randomly sub-sampled, with observers recording standard length, sex and collecting otoliths for each individual as part of the routine stock assessment and monitoring activities. Otoliths were aged from transverse sections using transmitted light at a magnification of  $12.5\times$  using a Leica MZ80 dissecting microscope using the established ageing protocols (see Kalish et al., 1996; Morison et al., 1998). The first 15 annual increments were marked along a ventral region adjacent to the sulcus acusticus and saved on each otolith image using custom software. Annual increments were composed of one translucent 'summer' and one

opaque 'winter' zone with marks placed on the outside edge of the opaque zone. To test the utility of the increments marked on the otolith image, pre-marked zone locations were regressed against a linear transect using the image analysis software package ImageJ (Rasband, 2013) and the ObjectJ plugin, (Vischer and Nastase, 2014). Otolith increment measurements precision and linearity was validated ( $F = 1037$ ,  $df\ 1744$ ,  $P < 0.001$ ) with the re-analyzed object J otolith increment data after log-log transformation of the data. Otolith increment data were recorded from the otoliths of *M. novaezelandiae* captured over a five-year period between 2009 and 2013 with a total of 7334 sectioned otolith samples being processed.

Standard length-at-age for 34860 individual fish caught between the years 1984–2013 were used for regional and sex based von Bertalanffy growth model development, Eq. (1). Where  $L_t$  is the length of a fish at age/time  $t$ ;  $L_\infty$  the asymptotic length;  $t_0$  is the theoretical age/time when length is equal to zero. The growth coefficient  $K$  is a measure of the rate at which the theoretical maximum size is attained.

$$L_t = L_\infty [1 - e^{-K(t-t_0)}]$$

Growth curves were generated for fish caught in four Tasmanian fishing zones, eastern Bass Strait (EBS), eastern Tasmania (ETAS), western Bass Strait (WBS) and western Tasmania (WTAS). Due to limited age ranges in some regions and to allow direct comparisons of models, data for modelling were restricted to fish 15 years of age and less.

The von Bertalanffy growth models were tested for significant differences in sex and capture zone using Analysis of Residual Sums of Squares (AoRSS) (Haddon, 2011). Following these analyses, all further tests were restricted to data from the WTAS fishing zone (standard lengths:  $n = 21759$ ; otolith sections:  $n = 4049$ ). Pair wise comparisons between sexes using T-tests were conducted on mean standard length, mean cumulative otolith increment widths- and age specific otolith increment widths. Effect size and direction was tested using Cohen D values, where values  $\leq 0.2$  are small/trivial, values between 0.2–0.8 are medium effect sizes and values above 0.8 are large effect sizes (Cohen, 1988). To further clarify between biological relevance and statistical effects due to large sample sizes, length-at-age, cumulative otolith increment widths and age specific otolith increment widths data were bootstrapped with replacement using a visual basic program in Excel (Haddon, 2011). The program generates a random sex specific subsample of the data with sample sizes of  $n = 1000$ , 100, 50 and 30 and conducts a T-test for each pair wise sex comparison at age. The output of each bootstrapped T-test was saved for each sample size grouping at age and repeated for 1000 cycles. Significant bootstrapped T-tests findings for each age bracket up to the age of 15 years were reported as a percentage of the total number of runs conducted. Due to the outcomes of the Cohen's D values and bootstrapping tests all remaining analyses on otolith increment measurements were conducted on pooled data.

### 2.2. Juvenile otolith growth and sex ratio

Inter-annual variation in juvenile cohort specific otolith growth patterns were investigated for the first five growth zones. Year-of-birth (YOB) was assigned by subtraction of the assigned age from the capture date for each individual. Specific sample descriptions for each YOB are detailed in Table 1. Inter-annual variability in mean otolith increment widths for each zone against YOB were analysed using one-way analysis of variance. Inter-annual variation in the sex ratio across capture years and YOB were also examined. Chi-squared tests were performed on the ratio of males to females based on capture year and cohort YOB. The influence of male offspring ratio per cohort on inter-annual otolith growth patterns was investigated using linear regression analysis for the first five growth zones. These statistical analyses were done using the statistical software 'R' (R Core Team, 2016).

**Table 1**

Macrurus novaezelandiae captured between 1984 and 2013 biological sampling description for year of birth (YOB) between 1976 and 2011.

YOB	Capture years	Male count	Female count	Otolith batches	Min Age	Max Age	Mean Age
1976	1984–1999	35	57	27	8	23	15.7
1977	1984–2000	90	96	42	7	23	14.6
1978	1984–2001	199	181	55	6	23	12.1
1979	1984–2000	282	220	60	5	21	9.9
1980	1984–2000	122	155	62	4	20	12.1
1981	1984–2003	169	145	70	3	22	10.8
1982	1984–2004	153	219	74	2	22	8.7
1983	1985–2006	195	181	81	2	23	12.1
1984	1986–2006	354	412	102	2	22	11.3
1985	1986–2008	446	474	121	1	23	11.5
1986	1991–2007	756	771	143	5	21	11.0
1987	1992–2009	803	756	141	5	22	10.3
1988	1992–2009	375	424	113	4	21	9.8
1989	1992–2009	139	246	94	3	20	9.9
1990	1993–2010	149	167	88	3	20	7.8
1991	1993–2013	184	157	88	2	22	6.6
1992	1993–2013	136	93	82	1	21	7.3
1993	1995–2013	458	325	169	2	20	10.2
1994	1995–2013	2486	3249	252	1	19	11.8
1995	1997–2013	1078	1225	217	2	18	9.2
1996	1998–2013	223	311	136	2	17	9.1
1997	1999–2013	64	88	78	2	16	9.9
1998	2001–2013	17	36	28	3	15	11.1
1999	2002–2013	12	19	21	3	14	10.8
2000	2003–2013	11	11	14	3	13	8.9
2001	2004–2013	29	22	30	3	12	9.2
2002	2006–2013	186	166	83	4	11	7.8
2003	2005–2013	1822	1575	141	2	10	6.7
2004	2006–2013	168	179	56	2	9	4.6
2005	2008–2013	42	39	29	3	8	6.0
2006	2008–2013	372	316	72	2	7	4.8
2007	2008–2013	116	91	30	1	6	3.6
2008	2009–2013	130	46	19	1	5	4.4
2009	2010–2013	119	41	16	1	4	3.7
2010	2012–2013	120	63	9	2	3	2.9
2011	2013–2013	22	21	5	2	2	2.0

### 3. Results

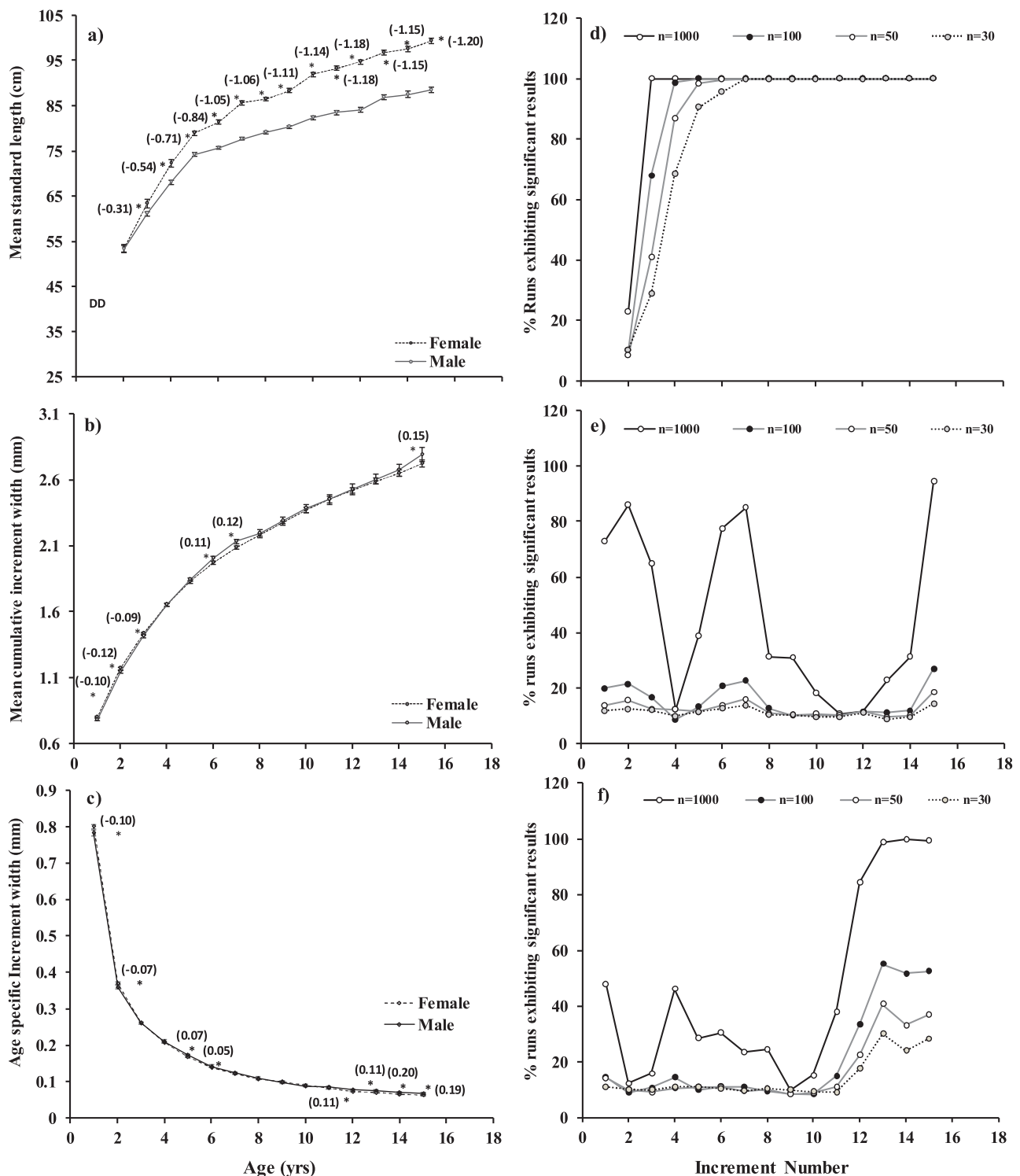
#### 3.1. Growth modelling and increment growth

Analysis of Residual Sum of Squares on von Bertalanffy growth models between male and female *M. novaezelandiae* indicated that growth was significantly different between sexes within each region and as a whole stock (Table 2). Growth model differences between fishing zones were also significantly different in all but two of the model comparisons. No significant differences were indicated in growth models between WBS and ETAS for male only and female only growth models (AoRSS: male:  $F = 1.81$ ,  $df$  3, 26,  $P > 0.05$ ; female:  $F = 1.34$ ,  $df$  3, 26,  $P > 0.05$ ).

Significant differences in mean standard length-at-age between sexes were first detected at age three, with females reaching larger sizes and the relative difference between mean size at age increasing with age (Fig. 1a). Bootstrapping these data showed a significant difference from the age of six in 95% of cycles even at low sample size ( $n = 30$ ) (Fig. 1b). Cohens D values were all negative (i.e. females were larger) and highlighted large effect sizes ( $> 0.8$ ) from the age of 5. In contrast, a diverging age, size, sex effect was not evident in cumulative otolith widths or age specific otolith increment widths. Significant differences between sexes were present in the mean cumulative otolith width-at-age for ages 1, 2, 3, 6, 7, and 15 years with all significant pair wise comparisons having small and variable direction Cohen D values  $\leq 0.15$  (Fig. 1c). A similar trends was also evident for sex specific otolith increment widths at age with significant differences present for the ages 1,2,5,6,12,13, 14 and 15, and all significant pair wise comparisons having small and variable direction Cohen D values  $\leq 0.2$  (Fig. 1e). Bootstrapping of otolith increment width data highlighted that these

**Table 2**Results of Analysis of Residual Sum of Squares (AoRSS) pairwise comparisons between growth models for male and female *M. novaezelandiae* caught in four Tasmanian fishing zones. Combined model parameters are presented for non-significant pairwise tests.

Variables	Pairwise test	P	n	Parameter 1			Parameter 2		
				$L_{\infty}$	K	$t_0$	$L_{\infty}$	K	$t_0$
All zones	Males-females	< 0.001	34860	87.5	0.3	−1.3	99.1	0.2	−1.1
EBS	Males-females	< 0.01	2361	90.4	0.3	−0.7	94.1	0.3	−0.4
ETAS	Males-females	< 0.001	2412	84.2	0.2	−1.6	98.2	0.1	−3.1
WBS	Males-females	< 0.001	7210	86.2	0.2	−1.8	93.5	0.2	−1.8
WTAS	Males-females	< 0.001	22877	89.0	0.2	−2.0	99.3	0.2	−1.1
Males	EBS-ETAS	< 0.001	2229	90.4	0.3	−0.7	84.2	0.2	−1.6
Males	EBS-WBS	< 0.001	5003	90.4	0.3	−0.7	86.2	0.2	−1.8
Males	EBS-WTAS	< 0.05	12577	90.4	0.3	−0.7	89.0	0.2	−2.0
Males	WBS-ETAS	> 0.05	5436	85.2	0.2	−1.7			
Males	WBS-WTAS	< 0.001	15784	86.2	0.2	−1.8	89.0	0.2	−2.0
Males	WTAS-ETAS	< 0.001	13010	89.0	0.2	−2.0	84.2	0.2	−1.6
Females	EBS-ETAS	< 0.001	2544	94.1	0.3	−0.4	98.2	0.1	−3.1
Females	EBS-WBS	< 0.001	4568	94.1	0.3	−0.4	93.5	0.2	−1.8
Females	EBS-WTAS	< 0.05	12661	94.1	0.3	−0.4	99.3	0.2	−1.1
Females	WBS-ETAS	> 0.05	4186	95.4	0.2	−2.4			
Females	WBS-WTAS	< 0.001	14303	93.5	0.2	−1.8	99.3	0.2	−1.1
Females	WTAS-ETAS	< 0.001	12279	99.3	0.2	−1.1	98.2	0.1	−3.1



**Fig. 1.** Comparisons of a) length at age ( $n = 21759$ ), b) cumulative increment width at age, or c) age specific increment width at age of male and female *M. novaezelandiae* caught in western Tasmanian fishing zone ( $n = 4049$ ) to 15 years. Error bars are  $\pm$  95% confidence intervals; \* indicate where  $t$ -test statistical differences were highlighted between sexes ( $p < 0.05$ ). Values in parentheses are Cohen D values where negative values indicate a higher female value. Absolute values less than 0.2 are small/trivial effects, medium effects  $< 0.8$  and large effect  $> 0.8$ . Percentage ( $n = 1000$  runs) of bootstrapped runs (random resampling with replacement) for d) standard length-at-age e) cumulative increment width at age, or f) age specific increment width at age returning significant  $t$ -test results between sex at each age with variable sample sizes.

statistical differences were due to large sample numbers with significant differences only observed in 95% of cycles at large sample size ( $n = 1000$ ) for ages above 13, for age specific otolith increment widths, and at the age of 15 for cumulative otolith widths at age (Fig. 1d and f). The sex specific effect direction for both cumulative otolith widths at age and age specific otolith increment widths were variable and

inconsistent with the observed sexual dimorphism for fish size. Due to the absence of biologically relevant and consistent differences in cumulative otolith increment widths-at-age or age specific increment widths between sexes, further analyses used pooled data.

**Table 3**

Tukey's HSD comparison of means between year of birth (YOB) and *M. novaezelandiae* juvenile increment growth. Groups with different lettering are significantly different.

Year of Birth	n	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
1993	83	a	bcd	abc	ab	b
1994	1300	ab	abcd	abc	b	b
1995	296	a	abc	abc	b	b
1996	96	ab	abcd	abc	b	b
1997	51	ab	abc	ab	b	b
1998	41	ab	ab	a	ab	b
1999	23	ab	abcd	abc	ab	ab
2000	15	abc	abcd	abc	ab	b
2001	41	ab	abc	abc	a	ab
2002	187	ab	abc	a	a	a
2003	1765	ab	a	a	ab	b
2004	149	ab	abc	ab	ab	b
2005	58	abc	a	ab	ab	b
2006	529	bc	abc	ab	ab	b
2007	161	cd	bcd	abc	ab	b
2008	132	e	bcd	bc	b	b
2009	135	e	d	c	b	
2010	150	e	cd	c		
2011	32	de	d			
df		18,5225	18,5221	17,5046	16,4731	15,4371
F statistic		58.8	15.4	15.3	11.6	9.2
p		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

### 3.2. Juvenile increment growth

Analysis of Variance for juvenile age specific otolith increment widths against YOB indicated highly significant inter-annual variations in mean increment widths for pooled sexes. This is particularly evident between 2006 and 2009 where a marked decrease in mean otolith increment width occurred in the first zone. The 2008–2010 cohorts recorded the lowest mean values for first year increment widths and were significantly lower from all years prior to 2008 (Table 3) (ANOVA:  $F = 58.8$ ,  $df$  18, 5225,  $P < 0.001$ ). Significant reductions in increment size were also evident after 2007 for zone 2 and after 2008 for zones 3 and 4 (Fig. 2).

### 3.3. Sex ratio

Annual sex ratios of males in the fishery ranged from 37.4 to 69.9% with an overall mean sex ratio of 50.3% (Fig. 3). Although no temporal trends were evident across capture years, sex ratios were significantly inter-annually variable (chi-squared tests for independence:  $\chi^2 = 642.8$ ,  $df$  22,  $P < 0.001$ ). The sex ratio within cohort birth years were also significantly inter-annually variable (chi-squared tests for independence:  $\chi^2 = 356.3$ ,  $df$  35,  $P < 0.001$ ) with the male ratio ranging from 32.1 to 74.4% with an overall mean sex ratio of 50.5%. The highest male dominated cohort biases were present in the years 2008–2010 (Fig. 4). Examination of the influence of male cohort ratios on first year otolith growth indicated that first year otolith growth was higher in female dominated cohorts and lower in male dominated cohorts. Linear regression showed a significant negative relationship with first year otolith increment widths-at-age and male cohort ratio (linear regression:  $F = 29.1$ ,  $df$  1,17,  $P < 0.001$ ; Fig. 5). This trend was not apparent in the remaining juvenile growth years.

## 4. Discussion

Results from this study highlight that *M. novaezelandiae* display clear sexual dimorphism with females attaining larger sizes. The divergence in length-at-age becomes detectable by the age of three. Female growth models in all four Tasmanian fishing regions showed higher asymptotic lengths ( $L_{\infty}$ ) than males. The rate at which  $L_{\infty}$  was reached ( $K$ ) was greater in males. Sexual dimorphism in the Australian

stock is well documented (Kailola et al., 1993; Smith, 1994). The current models for the four Tasmanian fishing regions differ from earlier growth models developed for WBS presented by Smith (1994). Smith (1994) reported modelled  $L_{\infty}$  for male *M. novaezelandiae* in WBS to be 95.5 cm and females 101 cm. The current study indicated  $L_{\infty}$  of WBS fish to have dropped to 86.2 cm and 93.5 cm for males and females respectively. In the other Tasmanian fishing regions  $L_{\infty}$  parameters were also lower than those reported by Smith (1994). Differences were also observed between  $K$  parameter values of the current study and those reported by Smith (1994) with values for males and females increasing to 0.25 and 0.23 from respective values of 0.2 and 0.18. It is important to note that the von Bertalanffy parameters  $K$  and  $L_{\infty}$  are not independent and this finding cannot be interpreted as evidence for increasing somatic growth rates as  $L_{\infty}$  is smaller in the current study. These contrasts in growth models could suggest a temporal shift in the life history characteristics of the species. Long term changes in growth rates have been reported for numerous exploited species from Eastern Australia, with the effect differing systematically with depth (Thresher et al., 2007; Ziegler et al., 2007). Decadal changes in life history parameters of Pacific halibut *Hippoglossus stenolepis* (Schmidt, 1904) in the north Pacific have been associated with a climate regime shift known as the Pacific Decadal Oscillation (Clark et al., 1999). Although the differences observed in this study are relatively small, continuation of such a trend would indicate that the overall maximum size of *M. novaezelandiae* could be decreasing, however, whether this may be due to intrinsic or extrinsic factors is unclear.

Historically growth modelling typically uses sex specific length-at-age data derived from multiple cohorts collected over time. This process pools inter-annual variability in growth which tends to have a smoothing effect with an associated loss of inter-annual differentiation. In recent years, many studies have utilised the analysis of otolith increment widths to investigate inter-annual variability in fish growth and/or sexual dimorphism (e.g. Akira et al., 2001; Villamor et al., 2004; Ito et al., 2007; Cross et al., 2009; Black et al., 2013; Doubleday et al., 2015; Morrongiello and Thresher, 2015). In many cases this approach assumes that trends in the otolith increment measurements are directly comparable to somatic growth between the sexes. This study suggests that this assumption needs to be viewed with caution. Decoupling of somatic growth with otolith increments has been reported in other species (Secor and Dean, 1989; Mugiya and Tanaka, 1992; Barber and Jenkins, 2001). Comparisons of length-at-age with cumulative otolith increment widths-at-age or age specific increment widths highlighted that otosomatic growth did not provide evidence of sexual dimorphism in *M. novaezelandiae*. Some sex differences at age in mean cumulative otolith increment widths and age specific increment widths were evident but the pattern was variable and not consistent with the observed somatic dimorphism of the species. It is important to note that due to the sensitivity of the  $t$ -test when large data sets are used, the statistical differences between age specific and cumulative otolith increment width values between genders was small and that the overall increment width size ranges were similar between sexes. A similar disconnect between the patterns exhibited in male and female somatic growth and cumulative otolith increment distances was also noted in a recent study of the southern blue spot flathead *Platycephalus speculator* Klunzinger 1872 otoliths (Haddy, unpublished). Morrongiello et al. (2012) suggested that due to the changing nature of somatic growth in fish, particularly post sexual maturity, careful consideration must be given in whether a true representation of somatic growth can be gained from otolith increment width analysis. While dimorphic somatic growth is evident in *M. novaezelandiae*, this study indicates that otosomatic growth in *M. novaezelandiae* is not influenced by sex. Therefore, caution needs to be exercised around the assumptions being made when using otolith increment data from sexually dimorphic species, especially if the data is being used for back calculation procedures or being interpreted as a proxy for somatic growth.

In the current study, only juvenile otolith increment data was

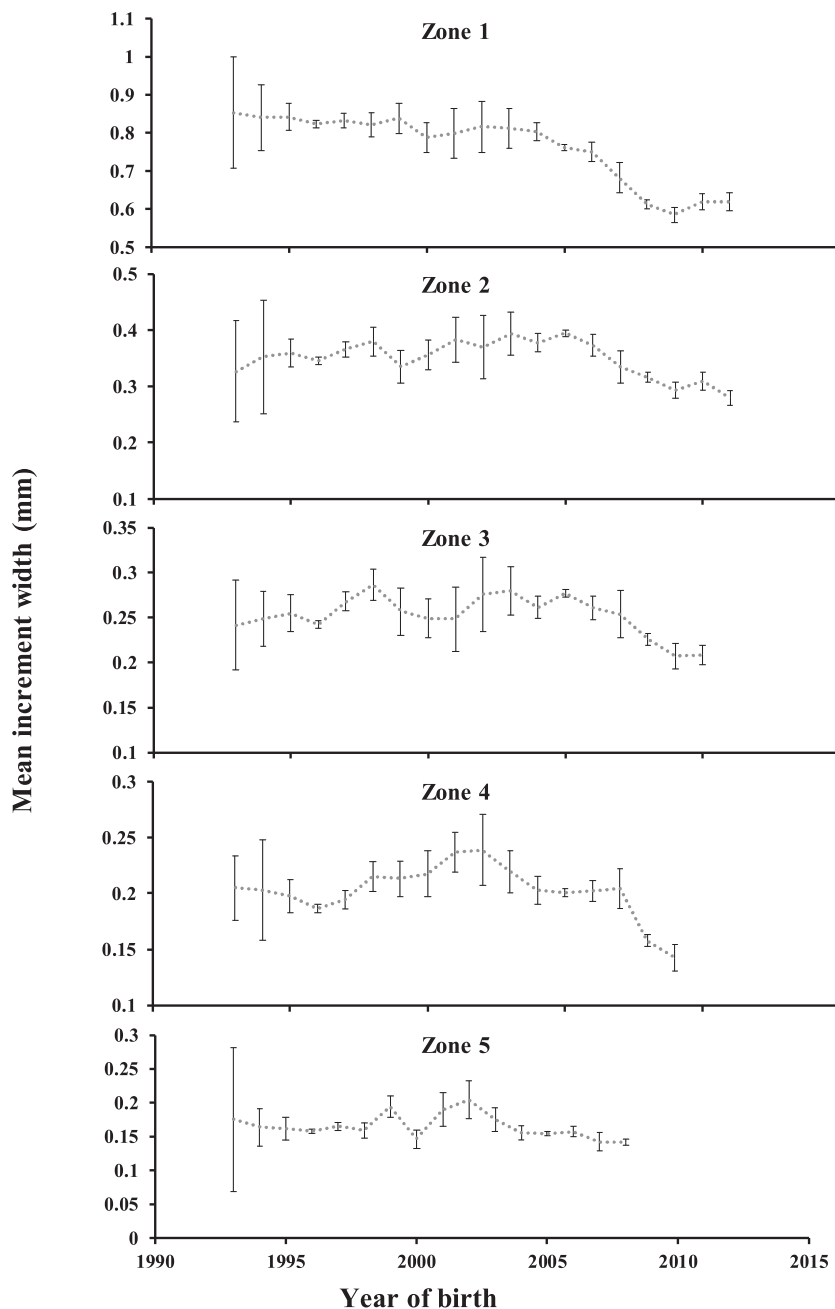


Fig. 2. Inter-annual growth variation in mean *M. novaezelandiae* increment widths-at-age up to the 5th growth zone for pooled sexes (error bars  $\pm$  95% CI). Table 3 presents post hoc ANOVA results for each zone. Note: y-axes scales vary to maintain visualisation of trend clarity.

investigated for inter-annual variation as the confounding effects of reproductive maturity and activity on growth could be isolated. Also, as increment widths are larger in juveniles the potential of observing environmental influences on otolith growth is increased. Other studies have suggested that restricting growth investigations to defined periods can enhance the accuracy and precision of results achieved (Thresher et al., 2007; Morrongiello et al., 2012). During the current study, inter-annual variability in juvenile otolith increment growth was evident in *M. novaezelandiae* juveniles. A declining trend in first year increment width commencing after 2006 and continuing through to 2010 was observed. The 2009 cohort was noted as the cohort with the narrowest juvenile growth zones except for the 2011 cohort first year growth which equalled the 2009 cohort. The trend of reducing increment widths during this period was also evident across other juvenile growth zones except for growth zone five. These results suggest that an unknown population wide event or combination of events have negatively impacted on juvenile otolith growth during this period.

Previous studies have reported density-dependent growth responses in *M. novaezelandiae* (e.g. Whitten et al., 2013). The declining trend indicated from the otolith increment data could indicate a density dependent effect as 2003 was a high recruitment year (Tuck, 2013). Density dependent growth has also been described for a number of fish species, for example in juvenile red drum *Sciaenops ocellatus* (Linnaeus 1766) by Bacheler et al. (2012); reduced juvenile growth at high densities and temperature in Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) in Crozier et al. (2010), in brown trout *Salmo trutta* Linnaeus 1758 by Baerum et al. (2013) and multiple species described in Lorenzen and Enberg (2002). As a similar response was not evident following the 1994 very high recruitment event, a density dependence effect is unlikely to be the sole contributor to the response. Environmental influences are well documented in influencing growth and are known to be reflected in otolith increment width measurements (Black et al., 2013; Doubleday et al., 2015). An index of upwelling events on the Tasmanian west coast presented by Kämpf (2015) demonstrates that

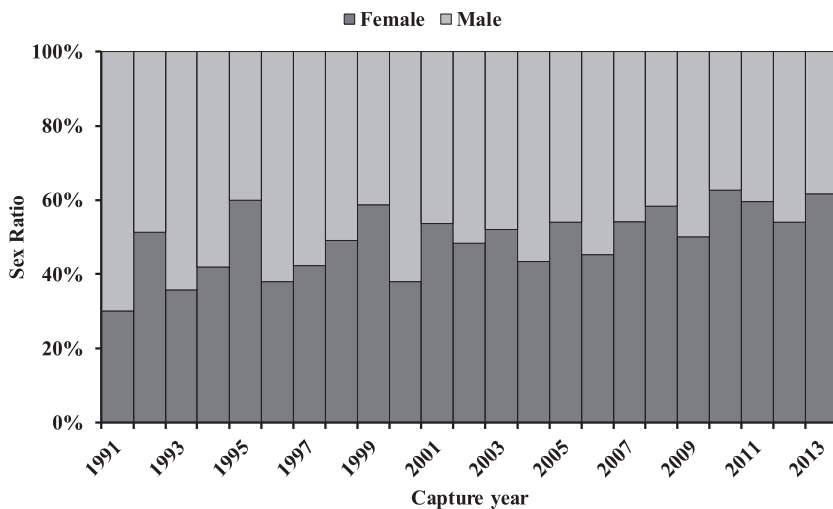


Fig. 3. Proportion of male and female *M. novaezelandiae* caught on the west coast of Tasmania for individual capture years ranging from 1991 to 2013.



Fig. 4. Proportion of male and female *M. novaezelandiae* offspring on the west coast of Tasmania for individual year-classes (cohorts) from 1976 to 2011, for sample details see Table 1.

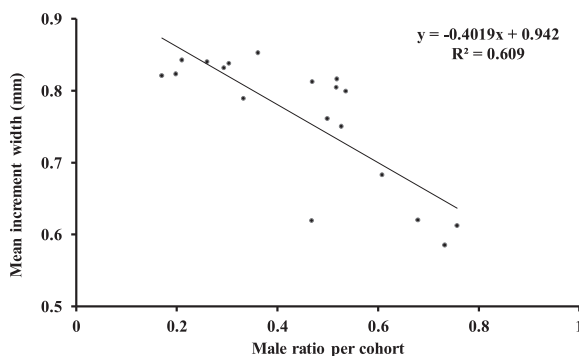


Fig. 5. The influence of increasing male ratios on mean *M. novaezelandiae* otolith increment widths during first year growth. Note: y-axis scale does not start at zero to allow visualisation of the data cloud.

during the spawning season down-welling favourable conditions typically occur but the strength index varies inter-annually. Although these strength index variations were not consistent with the progressive trends observed in otolith increment widths, the index also demonstrates that during 2005–2009 the durations of down-welling favourable conditions have progressively decreased with a subsequent increase in upwelling favourable conditions over the late spring to early autumn which is the major growth period for most fish. Upwelling events are commonly associated with high productivity, especially at the surface, but they also bring an influx of cold water up from the

benthic environments of the continental slope (Sommer et al., 2002; Nieblas et al., 2009). Temperature is well established as an influencing factor on growth rates in fish, with low temperatures known to reduce growth rates (Thresher et al., 2007; Pope et al., 2009). Black et al. (2013) demonstrated that otolith increment chronologies were strongly related to measured temperature at depth profiles in yellowfin sole, *Limanda aspera*, (Pallas, 1814). Unfortunately a similar analysis cannot be conducted in the present study due to a lack of definitive evidence on where the standing stock of juvenile *M. novaezelandiae* from the western Tasmanian fishery inhabit and a suitable time series of measured temperature at depth data for that location.

A major finding of the current study was demonstrating that cohort specific sex ratios display inter-annual variation. Although most of the literature investigating sex differentiation mechanisms in fish comes from an aquaculture context (Bezault et al., 2007), there is an increasing awareness that environmental sex determination systems exist in fish (Pandian, 2014). Environmental factors that have been associated with environmental sex determination include, temperature, hypoxia, pH, and/or endocrine disruptors (Pandian, 2014). A temperature dependent sex determination effect is the most common environmental sex determination system in fish and was first reported in Atlantic silverside *Menidia menidia* (Linnaeus 1766) (Conover and Kynard, 1981). Since then a temperature dependent sex determination influence has been claimed in over 59 species belonging to 13 families and four orders of fish (Pandian, 2014). The liable sex determination period in fish occurs from fertilisation to a critical time during early larval development prior to gonadal differentiation. The timing of gonadal differentiation has not been described for *M. novaezelandiae* but in Atlantic cod *Gadus morhua* Linnaeus 1758 and haddock *Melanogrammus aeglefinus* (Linnaeus 1758) it occurs at 90 days post hatch (dph) and 71 dph respectively (Chiasson et al., 2008).

The current study does not demonstrate that *M. novaezelandiae* is a temperature dependent sex determination species though such an effect could explain the inter-annual variation in cohort specific sex ratio observed. Further examination of the cohort specific sex ratio trends found that during 2004–2009, the ratio of female offspring in each birth year decreased. This sex ratio pattern was similar to the decreasing juvenile otolith growth pattern for the same period. When mean juvenile increment growth was considered in relation to the ratio of males present within a cohort, a significant negative relationship was evident. This could suggest that high levels of male progeny may negatively impact growth, however, the relationship was only present in the first-year growth zone, with cohort specific sex ratios having no effect on cohort specific otolith growth rates at other ages. This suggests that an environmental influence may be affecting both cohort specific sex determination and otolith growth rates. It is hypothesised that the

principal environmental driver of declining juvenile otolith growth performance and male biasing of cohort specific sex ratio is water temperature. For this to be addressed further, the location of where the standing stock of juveniles inhabit needs to be identified and a reliable time series of modelled/measured temperature at depth obtained from the area.

This study has highlighted aspects of *M. novaezelandiae* otolith growth which do not conform to normal expectations in accurately representing somatic growth between sexes. It has presented a novel approach to the examination of trends in populations and highlighted a pattern of inter-annual variability in otolith growth patterns and cohort specific sex ratios which may have long term consequences for the fitness of the population and corresponding flow-on effects into the stock assessment process and future management of the stock. It is recommended that further research is required into the associating potential environmental factors that can be associated with the observed inter-annual trends described in this study.

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